Impact of different fluoride concentrations and pH of dentifrices on tooth erosion/abrasion in vitro

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Abstract: BACKGROUND: This in vitro study analysed the effect of different fluoride concentrations in acidic or neutral liquid dentifrices in protecting enamel and dentine from erosive and abrasive wear. METHODS: Bovine enamel and dentine specimens (n = 132) were randomly allocated to 11 groups (each n = 12): experimental liquid dentifrices with 550 ppm F, 1100 ppm F, 5000 ppm F or 0 ppm F/placebo (each at pH 4.5 and pH 7.0); and commercial dentifrices with 550 ppm F (Colgate Baby, pH 7.0), 1100 ppm F (Crest, pH 7.0) and 5000 ppm F (Duraphat, pH 7.0). The specimens were subjected to erosion for 90 seconds, 4 times/day, over 7 days. Immediately after the first and last erosion, the specimens were brushed for 15 seconds using one of the dentifrices. Tooth wear was measured profilometrically (m) and analysed by ANOVA ($p < 0.05$). RESULTS: All fluoridated liquid dentifrices significantly reduced enamel wear compared to the placebo and commercial dentifrices. Only liquid dentifrices with 1100 and 5000 ppm F significantly reduced dentine wear compared to placebo dentifrice. The pH had no effect, but the consistency had a significant impact on the effect of dentifrices. CONCLUSIONS: Liquid dentifrices with high F concentration appear to be a good option to prevent tooth wear.

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Abstract

This *in vitro* study analysed the effect of different fluoride concentrations in acidic or neutral liquid dentifrices in protecting enamel and dentine from erosive and abrasive wear. Bovine enamel and dentine specimens (n=132) were randomly allocated to 11 groups (each n=12): experimental liquid dentifrices with 550 ppm F, 1100 ppm F, 5000 ppm F or 0 ppm F/placebo (each at pH 4.5 and pH 7.0); and commercial dentifrices with 550 ppm F (Colgate Baby, pH 7.0), 1100 ppm F (Crest, pH 7.0), and 5000 ppm F (Duraphat, pH 7.0). The specimens were subjected to erosion for 90 s, 4 times/day, over 7 days. Immediately after the first and last erosion, the specimens were brushed for 15 s using one of the dentifrices. Tooth wear was measured profilometrically (μm) and analysed by ANOVA ($p<0.05$). All fluoridated liquid dentifrices significantly reduced enamel wear compared to the placebo and commercial dentifrices. Only liquid dentifrices with 1100 and 5000 ppm F significantly reduced dentine wear compared to placebo dentifrice. The pH had no effect, but the consistency had a significant impact on the effect of dentifrices. Therefore, liquid dentifrices with high F concentration seem to be a good option to prevent tooth wear.

Keywords: Abrasion; Dentine; Enamel; Erosion; Fluoride Dentifrice

Introduction

Although the role of topical fluoride application on the prevention of tooth erosion is still controversial[1], the most recent research has focused on the effect of different fluoride compounds (NaF - sodium fluoride, AmF – amine fluoride, TiF_4 – titanium tetrafluoride, SnF_2 – stannous fluoride) and vehicles (varnish, gel, solution, dentifrice) to prevent tooth wear from erosion and abrasion[2].

Fluoridated dentifrices have been tested in several tooth wear studies, as dentifrice is already a widespread vehicle used for prevention of dental caries[3]. Although fluoridated dentifrices have the potential to decrease the development of tooth wear[4], recent studies have shown a limited beneficial effect of conventional 1100 ppm F dentifrices compared to non-fluoridated dentifrices on abrasion of eroded dentine and enamel, respectively[5,6].

Based on these findings, highly concentrated fluoride dentifrices (5000 ppm F) have been tested, but showed inconclusive results. In a recent *in situ* study, a commercial dentifrice with 5000 ppm F had the same positive effect as another commercial 1100 ppm F dentifrice on eroded and eroded-abraded dentine[7]. In respect to enamel, no significant differences were found between 0, 1100 and 5000 ppm F dentifrices on erosion and erosion-abrasion *in situ*[8]. Moretto et al.[9] produced contrasting data, showing that an experimental 5000 ppm F dentifrice was able to significantly reduce enamel erosion and erosion-abrasion compared to conventional 1100 ppm F dentifrice *in vitro*. Therefore, the effect of highly concentrated fluoride dentifrices on dental erosion and abrasion is still under debate.

On the other hand, the reduction of the pH and change of consistency (from paste to liquid or gel) has been shown to be effective in increasing plaque fluoride uptake *in vivo*[10]. Acidic dentifrices with 550 ppm F have the same effectiveness as neutral 1100 ppm F dentifrices in reducing enamel demineralisation *in vitro* and caries progression *in vivo*[11-13].

Although dentifrices with lower pH and higher fluid consistency than commercial dentifrices have been successfully employed in protocols involving carious lesions, they have never been tested in erosive-abrasive protocols. Their fluidity could increase reactivity with the dental substrate, while the low pH could enhance the tendency for calcium fluoride formation on apatite substrates[14]. Thus, the present *in vitro* study analysed the effect of different fluoride concentrations in acidic or neutral liquid dentifrices to prevent enamel and dentine erosive and abrasive wear.

The null hypotheses tested were as follows: (1) There is no significant difference in tooth wear between the liquid dentifrices with different F concentrations, regardless of the pH; (2) there is no significant difference in tooth wear between liquid dentifrices with different pH values, regardless of the F concentration; and (3) there is no significant difference in tooth wear between dentifrices with different consistencies (liquid versus paste or experimental versus commercial) that present the same F concentration and pH (neutral).

Materials and Methods

Preparation of the specimens

Enamel and dentine specimens (4 mm x 4 mm x 3 mm) were prepared from the labial surfaces of bovine incisor crowns and roots, respectively. The teeth were stored at 4°C in 0.1 % buffered thymol solution (pH 7.0) during this phase. The specimens were cut using an ISOMET low-speed saw (Buehler Ltd., Lake Bluff, IL, USA) with 2 diamond disks (Extec Corp., Enfield, CT, USA) separated by a 4-mm spacer. The specimens were ground flat with water-cooled silicon carbide discs (320-, 600-, and 1200-grade papers; Buehler, Lake Bluff, IL, USA) and polished with felt paper wet with diamond solution (1 μm; Buehler).

After polishing, the specimens were cleaned in an ultrasonic device with deionized water for 2 min. Prior to the experiment, 2 layers of nail varnish were applied to two-thirds of the surface of each specimen leaving a central test area and protecting reference surfaces for the profilometric measurement. The application of 2 layers of nail varnish assured that it was not rubbed off during the erosive and abrasive procedures. The specimens were maintained in 100 % humidity until the beginning of the experiment.

Twelve enamel and twelve dentine specimens were randomly allocated to each group: experimental liquid dentifrices with 550 ppm F, 1100 ppm F, 5000 ppm F and 0 ppm F/placebo (each at pH 4.5 and pH 7.0); and commercial dentifrices with 550 ppm F (Colgate Baby Barney, pH 7.0, Colgate-Palmolive, Brazil), 1100 ppm F (Crest, pH 7.0, Procter & Gamble, US), and 5000 ppm F (Prevident, pH 7.0, Colgate, US). A sample size of 12 specimens was calculated considering α-error level of 5 % and β-error level of 20 % based on previous data[7-9].

All dentifrices presented NaF as F compound with silica as abrasive. The composition of the liquid dentifrices is in accordance with a previous study[10]. The liquid dentifrices have the consistency of a gel, but with more fluidity. The RDA values of the experimental liquid dentifrices are displayed in Table 1.

Erosive and abrasive challenges

All specimens were submitted to 7-day erosion/abrasion cycles. Erosion was performed with freshly opened bottles of Sprite Zero (Coca-Cola Company Spal, Porto Real, RJ, Brazil, pH 2.6, 30 ml/specimen, unstirred, 25 °C), 4 times daily, for 90 s each. After demineralisation, the specimens were rinsed with tap water (5 s) and transferred into artificial saliva (pH 6.8, 30 ml/specimen, unstirred, 25 °C) for 2 h. After the last daily erosive treatment, the specimens were stored in artificial saliva overnight. The artificial saliva was renewed daily and consisted of 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2.2H_2O$, 3 mM NH_4Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K_2HPO_4, 3.3 mM urea, 2.4 mM NaH_2PO_4 and ascorbic acid (pH 6.8)[15].

All specimens were brushed twice daily with the dentifrices, immediately after the first (9 h) and the last (19 h) erosive challenges. The dentifrices were placed on the toothbrushes' heads (liquid dentifrices: ~0.15 g / commercial dentifrices: ~0.4 g) according to the study by Vilhena et al.[16] where the dentifrice weight applied on toothbrushes by children was measured. Then, the specimens were brushed using an electrical toothbrush (Colgate Motions Multi-action, Brazil) for 15 s (166 oscillations/s, 25 °C)[7,8]. The toothbrushes were fixed in a constructed device that allowed the heads to be aligned parallel to the specimens' surfaces. The toothbrushes' heads were weighed by using a precision scale (Pesola, Switzerland), and the weight was converted to force (1Kg~9.80665 N, F=1.5 N)[17]. The toothbrush heads were replaced daily. Thereafter, the specimens were rinsed in water for 5 s before being immersed in artificial saliva.

Profilometric measurement

Enamel and dentine wear (μm) was quantitatively determined by a contact profilometry (Mahr Perthometer, Göttingen, Germany) after the experiment. The specimens were maintained wet (100 % humidity) to avoid shrinkage of the dentine. For profilometric measurement, the nail varnish was carefully removed using a scalpel and acetone solution (1:1 water)[7,8]. The diamond stylus moved from the first reference to the exposed area and then over to the other reference area (2 mm long and 1.5 mm wide). Four profile measurements were performed for each specimen at intervals of 0.5 mm. The vertical distance between the horizontal line drawn on the reference areas and the horizontal line drawn on experimental area was defined as tooth wear using the software (Software Mahr Surf XT20, 2009). The values were averaged (µm) and submitted to statistical analysis. The standard deviation of repeated analysis of a given sample was 0.8 μm (mean wear of 10 μm).

Statistical analysis

Statistical analysis was performed with GraphPad InStat version 2.0 and GraphPad Prism software version 4.0 for Windows (Graph Pad Software, San Diego, CA, USA). The assumptions of equality of variances and normal distribution of errors were checked for all variables tested using Bartlett and Kolmogorov-Smirnov tests, respectively. Because the assumptions were satisfied, one-way ANOVA and Tukey's post hoc test (for comparison among all groups, separately for enamel and dentine, GraphPad InStat) and two-way ANOVA and Bonferroni post hoc test (for comparisons among the F concentrations, pH, and consistency, separately for enamel and dentine, GraphPad Prism) were applied. The significance level was set at 5 % (n=12).

Results

One-way ANOVA followed by Tukey's test revealed significant differences among the dentifrices for enamel and dentine, respectively ($p<0.01$, Tables 2 and 3).

Enamel

Two-way ANOVA (only for liquid dentifrices) revealed significant differences among the fluoride concentrations ($p<0.01$, $F=117.9$), but not between the pH values ($p>0.05$, $F=2.77$). There was no significant interaction between the factors. Bonferroni post hoc test showed that all fluoridated liquid dentifrices were able to similarly reduce enamel wear and were better than the placebo dentifrices.

These results were confirmed by the one-way ANOVA, which also showed no differences between the placebo dentifrices and commercial dentifrices (Table 2).

Regarding consistency, two-way ANOVA (liquid versus commercial dentifrices, including only dentifrices with the same F concentration and pH) revealed significant differences between the fluoride concentrations ($p<0.05$, $F=4.90$), as well as between the consistencies ($p<0.01$, $F=186.6$). There was no significant interaction between the factors. For all fluoridated dentifrices, consistency had a significant impact on their effect, i.e., the liquid dentifrice was more effective in reducing enamel wear than the commercial dentifrice.

Dentine

Two-way ANOVA (only for liquid dentifrices) also revealed significant differences among the fluoride concentrations ($p<0.01$, $F=10.42$), but not between the pH values ($p>0.05$, $F=1.71$). There was no significant interaction between the factors. Bonferroni post hoc test showed that liquid dentifrices with 1100 and 5000 ppm F were able to significantly reduce dentine wear compared to the placebo dentifrices.

Table 3 shows the differences between the dentifrices on dentine wear when ANOVA/Tukey's tests were applied. In this case, the commercial dentifrices presented the worst results.

Regarding the consistency, two-way ANOVA (liquid versus commercial dentifrices, including only dentifrices with the same F concentration and pH) revealed significant differences between the consistencies ($p<0.01$, $F=37.71$). In this case, the effect of F concentration was not significant ($p>0.05$, $F=0.68$), and the interaction between the factors was significant ($p<0.01$, $F=14.63$). The consistency had a significant impact on the effect of 1100 and 5000 ppm F: The liquid dentifrice was more effective in reducing dentine wear than the commercial dentifrice. However, consistency was not important in the case of the 550 ppm F dentifrice.

Based on the results, the null hypotheses were (1) rejected, (2) accepted, and (3) rejected.

Discussion

The action of conventional fluorides, such as NaF included in the experimental dentifrices, is mainly attributed to a precipitation of CaF_2-like material on tooth surfaces[18,19], which is able to partially reduce tooth wear by subsequent erosive-abrasive challenges[6-9]. This layer should behave as a physical barrier, hampering the contact of the acid with the underlying enamel, or as a mineral reservoir that is attacked by the erosive challenge, thus buffering the acids or promoting remineralisation[2].

Ganss et al.[19] evaluated the retention of calcium fluoride on human enamel and dentine under neutral and erosive conditions *in vitro* and *in situ*. It was shown that more calcium fluoride was lost under erosive compared to neutral conditions *in vitro*. Compared to the *in vitro* study, the CaF_2 precipitates were retained to a higher extent on the tooth *in situ*. Salivary proteins play a role in the solubility of CaF_2-like layers either by maintaining the supersaturated stage of saliva with respect to phosphate and calcium or by adsorption to the surface. In addition, the precipitates were less stable on dentine than on enamel under erosive conditions.

The formation of the CaF_2-like layer and its protective effect on demineralisation also depends on the pH and F concentration of the agent[20]. Regarding tooth abrasion, other important factors that should be considered are the type, size, and quantity of dentifrices' abrasives, which seem to be more important than the stiffness of the toothbrush filaments[21-23]. In the present study, all experimental dentifrices presented silica as abrasive with slight differences in the RDA value. Therefore, it is important to discuss the interaction among fluoride concentration, consistency (it might be related to the abrasivity), and pH of dentifrices on tooth wear. Accordingly, the present study showed that the F content and the consistency influence enamel and dentine wear, while the pH of the liquid dentifrices had no effect on tooth wear.

In the case of dentine, the best treatments to reduce wear were the liquid 1100 and 5000 ppm F dentifrices. On the other hand, Duraphat dentifrice, the commercial dentifrice with the highest F concentration, was least effective in preventing dentine wear. This result might be related to its higher abrasivity (RDA 77) compared to the experimental one (5000 ppm F, pH

7.0, RDA 69). This finding is not in agreement with a previous *in situ* study, in which the same commercial dentifrice with 5000 ppm F was better than a placebo (placebo formulation was different from the placebo dentifrice used in the present study) for the prevention of erosion and abrasion[7]. In the present study, the commercial dentifrice was applied undiluted on the dentine surface while in the previous *in situ* study, the dentifrice was diluted using water (slurry, 1:3). The application of the undiluted dentifrice directly on the surface might allow more fluoride in contact with the tooth, but it would also enhance the impact of the abrasive of the dentifrice compared to the slurry[24]. Additionally in the *in situ* condition, the abrasive challenges might be modulated by saliva and acquired pellicle.

Different from dentine, all experimental F dentifrices had some impact on enamel erosion as F precipitates are more stable on enamel than on dentine[19]. Regarding the commercial dentifrices, Rios et al.[8] could not demonstrate a preventive effect of 5000 ppm F (Duraphat) and 1100 ppm F (Crest) dentifrices on enamel erosion-abrasion *in situ*, in agreement with the present results. As the remaining softened layer of eroded enamel is considerably smaller compared to the amount of enamel wear, it might be speculated that the fluoride application predominantly prevents further erosive tissue wear by forming a mechanical barrier rather than by remineralising the softened surface. Furthermore, toothbrushing with conventional fluoridated dentifrices, which might be more abrasive than the experimental ones, might have completely removed this thin softened layer rather than allowed the remineralisation and the formation of CaF_2 precipitates.

On the other hand, Moretto et al.[9] showed a significantly higher preventive effect of an experimental 5000 ppm F compared to 1100 ppm F dentifrice on enamel erosion-abrasion *in vitro*. We could not see such difference between our experimental fluoridated dentifrices (5000 vs. 1100 ppm F). This finding might be related to the liquid consistency of our experimental dentifrices, which seems to have a remarkable influence on the tooth erosive and abrasive wear.

Confirming previous studies, fluoride seems to be more effective on enamel compared to dentine[2]. The fact that liquid dentifrices were better than the commercial ones confirms the hypothesis that consistency might also have an impact on the effect of the fluoridated dentifrice on tooth erosion-abrasion. It can be speculated that liquid dentifrices allow higher CaF_2 formation compared to commercial dentifrices. This speculation is based on a previous clinical study showing that liquid dentifrices enhanced the incorporation of fluoride on dental plaque compared to commercial dentifrices[10]. Furthermore, the RDA values were slightly higher for the commercial dentifrices compared to the experimental liquid ones.

Regarding the lack of effect of the acidic versus neutral pH of the liquid dentifrices as seen with the present protocol, our findings are in disagreement with previous publications that showed a beneficial effect of acidic dentifrices when used in cariogenic challenges[11,12]. On the other hand, Lussi et al.[25] showed no differences among commercial dentifrices with different pH ranges (pH 4.0–7.0) on the prevention of enamel erosion. Because erosive lesions occur in a pH range lower than the one associated with the occurrence of carious lesions, the reduction of the dentifrices pH might be unable to enhance the reaction between fluoride and eroded tooth surface, thus not improving its preventive effect against tooth wear.

Based on the results, it was concluded that the experimental liquid dentifrices, especially those with high fluoride concentrations, might be beneficial in reducing tooth wear by mild erosive and abrasive challenges *in vitro*. Further studies should be conducted *in vivo* to confirm the present findings. Such studies could also combine high concentrated fluoride dentifrices with other remineralising products such as CPP-ACP, which have shown some effect against erosion[26].

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Legends

Table 1. Mean ± S.D. of the RDA values of the dentifrices.

Table 2. Mean enamel wear (μm) ± SD of specimens subjected to erosion plus abrasion in the presence of different dentifrices.

Table 3. Mean dentine wear (μm) ± SD of specimens subjected to erosion plus abrasion in the presence of different dentifrices.

Table 1. Mean and S.D. of the RDA values of the dentifrices.

Dentifrices	**RDA***
550 ppm F, pH 4.5	56 ± 7[ab]
550 ppm F, pH 7.0	62 ± 6[bc]
1,100 ppm F, pH 4.5	70 ± 5[c]
1,100 ppm F, pH 7.0	52 ± 7[a]
5,000 ppm F, pH 4.5	67 ± 10[c]
5,000 ppm, pH 7.0	69 ± 10[c]
Placebo, pH 7.1	47 ± 4[a]
Placebo, pH 4.5	72 ± 5[c]
Commercial 550 ppm F	unknown
Commercial 1,100 ppm F	100**
Commercial 5,000 ppm F	77**

*Different letters show significant differences among the experimental dentifrices (one-way ANOVA and Tukey's test, $p<0.01$). ** The data were obtained from the Literature[7]

Table 2. Mean enamel wear (μm) ± SD of specimens subjected to erosion and abrasion in the presence of different dentifrices.

[F] (ppm)	Experimental Liquid Dentifrices	
	Acidic	Neutral
0	9.6±1.2[a]	9.8±1.0[a]
550	5.6±0.8[b]	6.4±0.4[b]
1,100	5.5±0.8[b]	5.5±1.2[b]
5,000	5.5±0.9[b]	5.7±1.0[b]
	Commercial Dentifrices	
	Acidic	Neutral
550	_____	9.3±1.0[a]
1,100	_____	8.8±0.9[a]
5,000	_____	9.8±0.8[a]

Different letters show significant differences among the dentifrices (one-way ANOVA and Tukey's test, p<0.01).

Table 3. Mean dentine wear (μm) ± SD of specimens subjected to erosion and abrasion in the presence of different dentifrices.

[F] (ppm)	Experimental Liquid Dentifrices	
	Acidic	Neutral
0	5.6±0.7^{cd}	5.3±0.4^{abc}
550	5.4±0.5^{bc}	5.3±0.8^{abc}
1,100	4.8±0.5^{ab}	4.8±0.6^{ab}
5,000	4.8±0.5^{ab}	4.6±0.6^{a}
	Commercial dentifrices	
	Acidic	Neutral
550	_____	5.2±0.5^{abc}
1,100	_____	5.8±0.5^{cd}
5,000	_____	6.4±0.8^{d}

Different letters show significant differences among the dentifrices (one-way ANOVA and Tukey's test, p<0.01).